BLAST+ now has a better BLAST database.

This document concerns some new BLAST features available in stand-alone BLAST. These changes do not affect the BLAST web interface.

Recent enhancements to BLAST+

We have made some recent enhancement to the BLAST+ applications that:

- 1.) Allows you to limit your search by taxonomy using information built into the BLAST databases.
- 2.) Have improved performance when limiting searches with an accession list.
- 3.) Can retrieve sequences by taxonomy from a BLAST database with blastdbcmd.

We've introduced a new version of the BLAST databases (version 5) to support the items listed above. You'll need to use BLAST+ 2.8.0 or later to take advantage of these new features. The default databases are also still at version 4, so we're providing version 5 copies of some popular databases.

This document provides information about using these new databases. First, we provide information on how to access the latest BLAST executables and the version 5 databases. Second, we provide some examples that demonstrate new features associated with the version 5 databases. Finally, we demonstrate how quickly these searches run.

Executables and Databases

You can download the latest version of the BLAST+ executables at https://ftp.ncbi.nlm.nih.gov/blast/executables/blast+/LATEST/

You can find version 5 databases at https://ftp.ncbi.nlm.nih.gov/blast/db/v5

Use update_blastdb.pl (included with the BLAST+ package) to download the version 5 databases, just use the --blastdb_version flag:

```
update_blastdb.pl --blastdb_version 5 --showall
Connected to NCBI
env_nr_v5
nr_v5
nr_v5
swissprot_v5
taxdb
tsa_nr_v5

update_blastdb.pl --blastdb_version 5 nr_v5 --decompress
Connected to NCBI
Downloading nr_v5 (36 volumes) ...
```

You will also need to install the EDirect command-line utility if you wish to look up TAXIDs via the command-line script, get_species_taxids.sh (see below). Instructions for installing EDirect are available at https://www.ncbi.nlm.nih.gov/books/NBK179288/ The shell script we provide will execute the actual EDirect commands. EDirect is only available for MacOSX and LINUX.

If you already have BLAST installed, make sure you are using the newly downloaded executables as well as the version 5 databases.

Examples

Limiting a search by taxonomy

In order to limit your BLAST+ search by taxonomy, you'll need to obtain the taxid(s) for your organism(s). A taxid is simply a number that specifies a node in the taxonomic tree. For example, 9606 is the taxid for human, 9989 is the taxid for rodentia, and 2 is the taxid for all bacteria. Taxids are preferable to organism names as the latter can be ambiguous. For example, bacteria is both a genus of insect as well as a superkingdom. BLAST will only accept taxids that are at or below the species level.

We provide a script to translate higher level taxids (e.g., Enterobacterales) into a list of taxids that are at the appropriate level (details below). The same script can also be used to used to lookup (and disambiguate) taxids based upon a taxonomic name. The script is called get_species_taxids.sh and is part of the BLAST+ package. As noted above, you will also need to install the EDirect command-line utility if you wish to use get_species_taxids.sh. Instructions are at https://www.ncbi.nlm.nih.gov/books/NBK179288/

You can limit your stand-alone BLAST+ search by specifying one or more (comma-delimited) taxids on the command-line, or you can specify a file containing multiple taxids. Some example command-lines:

Running a BLAST search with a higher level taxonomic node (Enterobacterales):

```
get_species_taxids.sh -n Enterobacterales

Taxid: 91347
  rank: order
  division: enterobacteria
  scientific name: Enterobacterales
  common name:

1 matches found

get_species_taxids.sh -t 91347 > 91347.txids

blastn -db nt -query QUERY -taxidlist 91347.txids -outfmt 7 -out OUTPUT.tab
```

This example uses the -taxidlist parameter which takes a file as input.

Running a BLAST search with a species level taxid, human (taxid 9606):

```
blastn -db nt -query QUERY -taxids 9606 -outfmt 7 -out OUTPUT.tab
```

If you are not sure whether your taxid is at the species level or lower (or covers all such cases), it is safe to run get_species_taxids.sh and feed the output to BLAST.

Additionally, you may use the -negative_taxids and -negative_taxidlist options to exclude sequences by TAXID from your search.

Limiting a search by a list of accessions

You may use a list of accessions to limit a search with both the version 4 and version 5 databases. For version 5, the accession list must be preprocessed before it can be used in a search. This process checks that the accessions appear to be real and produces a file optimized for use with BLAST. It is also possible to confirm that all the accessions are actually in your target database. The command lines below demonstrate the commands you will need.

```
blastdb_aliastool -seqid_file_in 9606.pacc  # 9606.pacc is a text file with protein accessions. This command produces a file called 9606.pacc.bsl
blastp -db nr -query QUERY -outfmt "7 std staxid" -seqidlist 9606.pacc.
bsl  # This command searches nr limited to the accessions in the file 9606.pacc.bsl
```

Additionally, you may use the -negative_segidlist option to exclude sequences by accession from your search.

Faster sequence lookups by accession

The version 5 databases use LMDB (<u>Lightning Memory-Mapped Database</u>) to quickly retrieve sequences by accession. You can still use blastdbcmd for the retrieval and the old parameters are still supported (examples below). There are two new parameters (-taxids and-taxidlist) to retrieve sequences by taxid, and the next example demonstrates the usage of the -taxids parameter.

```
blastdbcmd -db nt -entry u0001
blastdbcmd -db nt -entry_batch FILE_WITH_ACCESSIONS
blastdbcmd -db nr -taxids 9606 -outfmt "%a %T %S" -target_only
# retrieves all human entries
# %a prints the accession, %T prints the taxid, %S prints the scientific name
```

-target_only is used with the last blastdbcmd command to ensure that only accessions for human entries are presented. Otherwise, it will present all accessions on any sequence with at least one human accession. This is important since nr is a non-redundant database.

Speed of searches

We search AAC51230 (human MEN1 protein) against nr restricted to human proteins by various means and once without any restriction. All searches were run three times in a row and the lowest time was selected.

Restrict nr by	Runtime (seconds)
TaxID	11.7
GI list	13.2
Accession list (version 5)	12.1
Accession list (version 4)	18.6
No restriction	2,865.0

Problems/Feedback

Please send problems reports or feedback to blast-help@ncbi.nlm.nih.gov or BLAST support.